

WHAT IS CLAIMED IS:

1. A method of separating components having a given negative or positive charge and contained in a sample, comprising
 - 5 loading a separation microchannel having, in an upstream to downstream direction, an upstream channel region, a sample-volume channel region, and a downstream separation channel region, so as to fill the upstream channel region with a trailing-edge electrolyte containing a selected concentration of a titratable species, the sample-volume channel region, with the dilute sample, and the separation channel region, with a leading-edge electrolyte,
 - 10 creating an electrical field potential across said channel, by applying a voltage potential across electrodes in contact with said upstream and downstream channel ends,
 - 15 by said applying, initially causing charged components in said sample to stack by isotachophoresis, and subsequently causing hydroxyl or hydrogen ions to migrate into the trailing-edge electrolyte, titrating said titratable species therein, under conditions that permit the sample to stack into a relatively small sample volume before hydroxyl- or hydrogen-ion migration into and through the sample-volume region is effective to overtake the charged sample components,
 - 20 wherein continued application of an electric potential across the channel ends causes charged sample components in the stacked sample volume to separate by zone electrophoresis.
- 25 2. The method of claim 1, wherein said upstream channel region includes a pair of upstream reservoirs, one containing the trailing-edge electrolyte, and the other containing a source of said hydroxyl or hydrogen ions, and said creating includes initially applying a voltage potential across electrodes in contact with said one upstream reservoir and the downstream channel end, and subsequently, applying a voltage potential across electrodes in contact with said other upstream reservoir and the downstream channel end.

3. The method of claim 1, wherein the upstream channel region is filled with a trailing-edge electrolyte containing a selected concentration of a titratable species, and said applying is effective cause charged components in said sample to stack by isotachophoresis, and, at the same time, electrolytic hydroxyl or hydrogen ions formed by electrolysis at the upstream-end electrode to migrate into the trailing-edge electrolyte, titrating said titratable species therein, where the concentration of said titratable species in the trailing-edge electrolyte is selected, in relation to the lengths of the upstream channel region and sample-loading volume, to permit the sample to stack into a relatively small sample volume before electrolytic-ion migration from the upstream electrode into and through the sample-volume region is effective to overtake the charged sample components, wherein continued application of an electric potential across the channel ends causes charged sample components in the stacked sample volume to separate by zone electrophoresis.

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4. The method of claim 3, wherein the trailing-edge electrolyte includes a trailing-edge ion and a titratable counter-ion buffer at said selected concentration.

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5. The method of claim 4, wherein the electrolytic ions formed at the upstream-end electrode are hydroxyl ions, and the titratable counter-ion buffer is a TRIS buffer.

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6. The method of claim 1, for use in detecting charged sample components present at nanomolar concentrations or less, wherein the ratio of sample volume before and after isotachophoretic stacking is at least about 10:1.

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7. The method of claim 6, wherein the ratio of sample volume before and after isotachophoretic stacking is at least about 50:1.

8. The method of claim 7, for use in detecting charged sample components present at picomolar or less concentrations, wherein the ratio of sample volume before and after isotachophoretic stacking is at least about 100:1.

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9. The method of claim 1, wherein the ratio of the lengths of the sample-volume region to the separation channel is between about 1:50 to 1:1.

10 10 The method of claim 1, wherein said ratio is between about 1:10 to 1:2.

15 11. The method of claim 3, wherein the leading-edge electrolyte contains a negatively charged leading-edge ion having an effective conductivity greater than that of the sample ions and a concentration between 1-50 mM, the trailing-edge electrolyte contains a negatively charged trailing-edge ion having an effective conductivity lower than that of the sample ions and a concentration of between 1-50 mM, and both electrolytes have a positively charged buffer at a selected concentration between about 2 and 50 mM.

20 12. The method of claim 1, wherein the separation microchannel is part of a channel network in a microfluidics device, which also includes first and second side channels which intersect the separation channel at axially spaced intersections, defining said sample-volume region between said intersections, and said loading includes moving said sample from a sample reservoir 25 connected to the first side channel through the sample-volume region and into the second side channel.

30 13. The method of claim 1, for use in separating a plurality of electrophoretic tags contained in a sample, each tag having a detectable moiety and a mobility modifier that confers on the tag, a unique electrophoretic mobility, which further includes the steps, after separating the tags electrophoretically, of

detecting the separated tags, and determining from their electrophoretic mobilities, the identify of probe from which the tags were cleaved.

14. The method of claim 13, wherein said tags are contained on
5 branched polymer structures and linked thereto through photo-labile linkages, and said method further includes, after permitting branched structures in the sample to stack into a relatively small sample volume, irradiating the branched structures to release the tags therefrom, wherein continued application of an electric potential across the channel ends causes said tags in the stacked
10 sample volume to separate by zone electrophoresis.

15. A microfluidics system for use in electrophoretic separation of components having a given negative or positive charge and contained in a dilute sample, comprising

15 (a) a microfluidics device having
 (i) a substrate,
 (ii) formed in the substrate, a channel network having a separation channel and first and second side channels that intersect the separation channel at axially spaced positions therealong, partitioning the separation microchannel, 20 in an upstream to downstream direction, into an upstream channel region upstream of intersection with the first side channel, a sample-volume channel region between the intersections of the two side channels, and a downstream separation channel region downstream of the second side channel intersection, where the ratio of the lengths of the sample-volume region to the separation
25 channel is between about 1:50 to 1:1,
 (iii) upstream and downstream reservoirs communicating with the upstream and downstream ends of said separation channel, respectively,
 (iv) first and second reservoirs communicating with the first and second side channels, respectively, opposite the side channel intersections with
30 the separation channel,
 (v) upstream and downstream electrodes adapted to contact liquid contained in the upstream and downstream reservoirs, respectively, and

(b) a control unit having a power source for applying a voltage potential across the upstream and downstream electrodes, under conditions such that, with the upstream channel region filled with a trailing-edge electrolyte, the sample-volume channel region filled with the dilute sample, and the separation channel region filled with a leading-edge electrolyte, the sample stacks into a relatively small sample volume before hydroxyl- or hydrogen-ion migration into and through the sample-volume region is effective to overtake the charged sample components, wherein continued application of an electric potential across the channel ends causes charged sample components in the stacked sample volume to separate by zone electrophoresis.

16. The system of claim 15, wherein said upstream channel region includes a pair of upstream reservoirs, one containing the trailing-edge electrolyte, and the other containing a source of said hydroxyl or hydrogen ions, and said control unit is operated to initially apply a voltage potential across electrodes in contact with said one upstream reservoir and the downstream channel end, and subsequently, to apply a voltage potential across electrodes in contact with said other upstream reservoir and the downstream channel end.

17. The system of claim 15, wherein the upstream channel region is filled with a trailing-edge electrolyte containing a selected concentration of a titratable species, and said applying is effective cause charged components in said sample to stack by isotachophoresis, and, at the same time, electrolytic hydroxyl or hydrogen ions formed by electrolysis at the upstream-end electrode to migrate into the trailing-edge electrolyte, titrating said titratable species therein, where the concentration of said titratable species in the trailing-edge electrolyte is selected, in relation to the lengths of the upstream channel region and sample-loading volume, to permit the sample to stack into a relatively small sample volume before electrolytic-ion migration from the upstream electrode into and through the sample-volume region is effective to overtake the charged sample components, wherein continued application of an electric potential

across the channel ends causes charged sample components in the stacked sample volume to separate by zone electrophoresis.

18. The system of claim 15, for use in detecting charged sample
5 components present at nanomolar concentrations or less, wherein the ratio of the lengths of the sample-volume region to the separation channel in said device is between about 1:50 to 1:1.

19. The system of claim 15, which includes one of a plurality of different
10 microfluidics devices having different channel-length ratios between 1:50 and 1:1, and said control unit is operable to calculate the approximate concentration of charged buffer ion in the trailing -ion buffer required for any selected microfluidics device length ratio.

15 20. The system of claim 15 wherein the control unit is operable to load (i) the downstream channel region with the leading-edge electrolyte, by applying an electrokinetic voltage across the downstream reservoir and one of the first and second reservoirs, (ii) the upstream channel region with the trailing-edge electrolyte, by applying an electrokinetic voltage across the upstream reservoir and one of the first and second reservoirs, and (iii) the sample volume region by applying a fluid-motive force effective to move sample contained in one of the first and second reservoirs through the sample-volume region and toward the other of the first and second reservoirs.
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25 21. The system of claim 20 wherein the microfluidics device includes first and second electrodes adapted to contact liquid contained in the first and second reservoirs, respectively, and said control unit is operable to load the sample volume region by applying an electrokinetic voltage across the first and second electrodes.

30 22. The system of claim 15 wherein said control unit is operable to apply across the upstream and downstream electrodes, a voltage potential characterized by a constant current, a constant voltage or constant power.